METABOLIC STABILITY OF SOME OXYTOCIN ANALOGUES IN HOMOGENATES OF RAT KIDNEY AND LIVER

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A study was made of the metabolic stability of certain oxytocin analogues in rat liver and kidney homogenates. The half-life of the individual analogues in tissue homogenates increased in the following series: oxytocin < deamino-oxytocin < deamino-carba-1-oxytocin < carba-1-oxytocin < tocinamide < deamino-tocinamide. Structural modifications of the oxytocin molecule that eliminated the possibility of aminopeptidase action and enzymic cleavage of the linear chain of the peptide molecule proved to be most effective in producing metabolically stable analogues.

Tocinamide and deamino-tocinamide (derivatives of oxytocin and deamino-oxytocin lacking the linear tripeptide) have a relatively high antidiuretic effect[1]. Tocinamide has protracted antidiuretic action in rats[2]. These findings led us to investigate the metabolic stability of the peptides in rat tissue homogenates. The studies also aimed at elucidating the mechanism of oxytocin inactivation in tissues. The liberation of terminal glycineamide[3] or leucylglycineamide[4] from the linear chain of the peptide molecule, which is seen to be one of the most important mechanisms of oxytocin inactivation in tissues, raises the question of the extent to which the two oxytocin analogues lacking the linear tripeptide are inactivated in tissues. The rate of tocinamide and deamino-tocinamide inactivation was compared with that of oxytocin, deamino-oxytocin, carba-1-oxytocin and deamino-carba-1-oxytocin. The latter three analogues are resistant either to aminopeptidase cleavage or the reduction of the disulphide bridge, or to both processes.

EXPERIMENTAL

*Material.** Oxytocin, deamino-oxytocin[5], carba-1-oxytocin[6] and deamino-carba-1-oxytocin[7] were prepared by Dr K. Jošt of the Department of Organic Synthesis of this Institute. Tocinamide[8] and deamino-tocinamide[9] were synthetized by Dr M. Zaoral of the above-mentioned department.

Liver and kidney homogenates. Female rats of the Wistar-Konárovice strain, weighing 180 to

* *Abbreviations*: carba-1-oxytocin, [6,1-β-cystathionine]oxytocin; deamino-carba-1-oxytocin, [6,1-β-deaminocystathionine]oxytocin; tocinamide, oxytocin-(1—6)-hexapeptide amide; deamino-tocinamide, deamino-oxytocin-(1—6)-hexapeptide amide.

200 g, were killed by decapitation, the two organs were excised and homogenized at 1—3°C in a solution of 1.5 mM-$MgCl_2$, 10 mM-Tris-HCl, 0·001 mM-EDTA; pH 7·2, using a Potter - Elvehjem homogenizer. The homogenates (approximately 20% w/v) were filtered through gauze and used immediately for incubation.

Incubation. Oxytocin and its analogues were incubated with aliquots of homogenates for 0—120 min at 37°C. The incubation mixture had the following composition: 1 μM peptide, 50 mM sodium phosphate buffer — pH 7·4 and 0·3—0·8 mg of protein nitrogen/ml of incubation mixture. The reaction was terminated by heating for 3 min in boiling water.

Uterotonic assay. The residual hormone activity was determined on the isolated rat uterus according to Munsick[10]. Reference samples for the assay were prepared by heating the incubation mixture containing the protein material for 3 min before adding the peptides.

The protein nitrogen content was determined in aliquots of the homogenates using the Kjeldahl method.

RESULTS AND DISCUSSION

With the exception of deamino-tocinamide, all the oxytocin analogues studied were inactivated in liver and kidney homogenates. The results are presented in Table I. The figures express the relative values of the half-life of the individual peptides. Oxytocin had the highest rate of inactivation; its mean half-life is placed equal to 1. It can be seen that the deamination of cystein in position 1 of the oxytocin peptide chain results in an increase of the metabolic stability of the analogue. Furthermore, the elimination of the reversible opening of the twenty-member ring by modifying the thioether bond also increases the stability of the analogue (carba-1-oxytocin). It is interesting that the combination of both modifications, *i.e.* deamination and substitution of sulphur by a methylene group, does not result in an analogue with a correspondingly longer half-life. Deamino-carba-1-oxytocin, although more stable

TABLE I

Rate of Inactivation of Oxytocin and Its Analogues in Rat Liver and Kidney Homogenates

The figures express the relative values of the half-life of the individual peptides. The mean half-life of oxytocin is placed equal to 1. The results are mean values of four experiments.

Compound	Homogenate	
	liver	kidney
Oxytocin	1·0	1·0
Deamino-oxytocin	5·95	2·7
Deamino-carba-1-oxytocin	8·9	5·7
Carba-1-oxytocin	16·6	7·5
Tocinamide	70·0	10·0
Deamino-tocinamide	∞	∞

than oxytocin[11], is inactivated more rapidly by the enzymes in tissue homogenates than carba-1-oxytocin. It is quite possible that the rate of inactivation is influenced by differences in the spatial conformation of the individual analogues which might make additional bonds of the peptide susceptible to enzymic cleavage.

Tocinamide, which contains the N-terminal amino group and an intact disulphide bridge but lacks the linear tripeptide, is inactivated at a significantly lower rate in both tissue homogenates than the other analogues studied. This finding can serve as indirect evidence that the cleavage of peptide bonds in the linear peptide chain of oxytocin may be the main inactivation pathway in animal tissues. Deamino-tocinamide, which by contrast to tocinamide has no N-terminal amino group, was most stable of the analogues studied. No inactivation was observed during 90 min of incubation with the homogenates. This may also be taken as indirect proof that oxytocin is mainly inactivated by endopeptidase cleavage of the linear chain of the molecule, and by aminopeptidase cleavage preceded by reversible opening of the peptide ring through reduction of the disulphide bridge. It is interesting to note in this context that an analogue of deamino-dicarba-oxytocin with glycine instead of proline in position 7 of the peptide chain has a strong affinity to the uterine oxytocin receptor, at the same time being remarkably resistant to enzymic cleavage[12].

The solving of the problem of the stability of oxytocin analogues in animal tissues opens the way to the next phase of research, namely the preparation of metabolically stable analogues with higher affinity to the individual receptors in target tissues.

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